SANTA CRUZ BIOTECHNOLOGY, INC.

GGA2 siRNA (m): sc-41170

BACKGROUND

A family of proteins, the GGAs (Golgi-localized, γ-adaptin ear-containing, ARF-binding proteins) sequences that showed significant homology to the carboxy-terminal "ear" domain of γ-adaptin. Members of the GGA family (GGA1, GGA2 (also known as VEAR or VHS domain and ear domain of γ-adaptin) and GGA3) are ubiquitous coat proteins that facilitate the trafficking of proteins between the *trans*-Golgi network and the lysosome. However, unlike γ-adaptin, the GGAs are not associated with clathrin-coated vesicles or with any of the components of the AP-1 complex. GGA1 and GGA2 are also not associated with each other, although they colocalize on perinuclear membranes. GGA2 shares 45% amino acid sequence identity with GGA1 and 35% with GGA3. In addition to being involved in heterotypic vesicle/suborganelle interactions associated with the Golgi complex, GGA2 may have a tissue-specific function and is highly expressed in kidney, muscle and heart. Furthermore, the VHS domain of GGA2 binds to the acidic cluster-di-leucine motif in the cytoplasmic tail of the cation-independent mannose 6-phosphate receptor (CI-MPR) and this is important for lysosomal enzyme targeting.

REFERENCES

1. Hirst, J., et al. 2000. A family of proteins with γ-adaptin and VHS domains that facilitate trafficking between the *trans*-Golgi network and the vacuole/lysosome. J. Cell Biol. 149: 67-80.
2. Poussu, A., et al. 2000. Vear, a novel Golgi-associated protein with VHS and γ-adaptin "ear" domains. J. Biol. Chem. 275: 7176-7183.
3. Nielsen, M.S., et al. 2001. The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. EMBO J. 20: 2180-2190.
4. Zhu, Y., et al. 2001. Binding of GGA2 to the lysosomal enzyme sorting motif of the mannose 6-phosphate receptor. Science 292: 1716-1718.
5. He, X., et al. 2002. Memapsin 2 (β-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2. FEBS Lett. 524: 183-187.
6. Zhu, G., et al. 2003. Crystal structure of GGA2 VHS domain and its implication in plasticity in the ligand binding pocket. FEBS Lett. 537: 171-176.

CHROMOSOMAL LOCATION

Genetic locus: Gga2 (mouse) mapping to 7 F2.

PRODUCT

GGA2 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GGA2 shRNA Plasmid (m): sc-41170-SH and GGA2 shRNA (m) Lentiviral Particles: sc-41170-V as alternate gene silencing products.

For independent verification of GGA2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41170A, sc-41170B and sc-41170C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GGA2 siRNA (m) is recommended for the inhibition of GGA2 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

GGA2 (E-3): sc-133147 is recommended as a control antibody for monitoring of GGA2 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GGA2 gene expression knockdown using RT-PCR Primer: GGA2 (m)-PR: sc-41170-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.